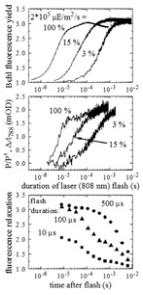
2796-Symp
Fluorescence Assays for Photosynthetic Capacity of Bacteria
Péter Maróti, Emese Asztalos, Gábor Sipka.
University of Szeged, Szeged, Hungary.
The fast kinetics of induction and relaxation of bacteriochlorophyll prompt and delayed fluorescence together with absorption changes of the reaction center (RC) dimer (P) were measured by combination of flashes from laser diodes in intact cells of wild type, carotenoidless (R-26) and cytochrome c_2 deficient (CYCA) mutants of photosynthetic bacteria *Rhodobacter sphaeroides*. The fluorescence induction under high intensity of continuous light splits into fast and slow rises both overlapped by the (carotenoid and/or bacteriochlorophyll) triplet quenching. The fast phase is purely photochemical as it depends strongly on the number of photons absorbed. The slow phase is the combination of thermal and photochemical reactions and reflects the multiple turnover of the system. Upon short flash, the fluorescence yield cannot reach the maximum due to partial reopening of the RCs by rapid donor and acceptor side reactions. Longer flashes are needed to close the RC completely. Contrary to higher plants, the kinetics of induction and relaxation of the fluorescence yield in bacteria are controlled principally by P^+. The reactions on the quinone side play minor role. The quantitative determination of the cyclic electron transfer rate can be based on calibration to the quantity of P^+.



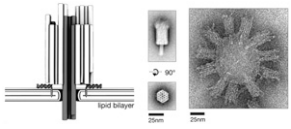
2797-Symp
Design and Engineering of a Light-Activated Potassium Channel
Cristian Cosentino[1], Gerhard Thiel[2], **Anna Moroni, Ph.D**[3].
[1]University of Milan, Milan, Italy, [2]TU-Darmstadt, Darmstadt, Germany, [3]biology, University of Milan, Milan, Italy.
Optogenetics uses light-activated ion channels to manipulate electrical signals of cells with high spatio-temporal precision. The available palette of optogenetics tools so far includes activators and inhibitors. The sodium permeant channelrhodopsin-2 for example depolarizes the membrane and increases excitability. Light-activated chloride and proton pumps function as inhibitors of excitability because they hyperpolarize the cell. To widen the repertoire of optogenetic tools, the search is on for a light-activated potassium (K^+) channel, which would enrich the efficacy of inhibitory switches. In the present work, we will describe our strategy to engineer, by a synthetic approach, a genetically encoded light-activated K^+ channel. To this end, we have fused the LOV (light oxygen voltage) domain of the plant blue-light receptor phototropin, to the viral K^+ channel Kcv. The functional properties of the resulting chimeric channels BLINK (blue light induced K^+ channel) were optimized by a combination of rational and irrational design using a high-throughput yeast-based screening system. We have so far selected two promising prototype channels, which show opposite regulation by blue light:, BLINK1 is activated, while BLINK2 inhibited by blue light. Both prototype channels are functional when expressed in Xenopus oocytes and we will discuss their biophysical properties in the context of future optogenetics applications.

Platform: Micro- and Nanotechnology

2798-Plat
DNA-Templated Silver Nanoclusters that Fluoresce upon Hybridization
James Werner[1], Jennifer S. Martinez[1], Jaswinder K. Sharma[2], Hsin-Chih Yeh[3].
[1]Los Alamos National Laboratory, Los Alamos, NM, USA, [2]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [3]University of Texas, Austin, TX, USA.
DNA-templated silver nanoclusters (DNA/Ag NCs) are an emerging set of fluorophores that are smaller than semiconductor quantum dots and can have better photostability and brightness than commonly used organic dyes. Here we find the red fluorescence of DNA/Ag NCs can be enhanced 500-fold when placed in proximity to guanine-rich DNA sequences, termed enhancer sequences. On the basis of this new phenomenon, we have designed a DNA detection probe (NanoCluster Beacon, NCB) that ''lights up'' upon target binding. Since NCBs do not rely on Förster energy transfer for quenching, they can easily reach high (>100) signal-to-background ratios (S/B ratios) upon target binding. Here, in a separation-free assay, we demonstrate NCB detection of an influenza target with a S/B ratio of 175, a factor of 5 better than a conventional molecular beacon probe. In addition, we show the fluorescence emission color of a NCB can change substantially (a shift of 60-70 nm in the emission maximum) depending upon the alignment between the silver nanocluster and the DNA enhancer sequence. We have exploited this color shift to directly detect single nucleotide polymorphisms (SNPs). This SNP detection method has been validated on all single-nucleotide substitution scenarios in three synthetic DNA targets, in six disease-related SNP targets, and in two clinical samples taken from patients with ovarian serous borderline tumors. Since the observed fluorescence enhancement is caused by intrinsic nucleobases, our detection technique is simple, inexpensive, and compatible with commercial DNA synthesizers.

2799-Plat
The Lawnmower: An Autonomous Synthetic Protein Motor
Laleh Samii[1], Suzana Kovacic[1], Cassandra Niman[2], Heiner Linke[2], Dek Woolfson[3], Paul M.G. Curmi[4], Martin J. Zuckermann[1], Nancy R. Forde[1].
[1]Simon Farser University, Burnaby, BC, Canada, [2]Lund University, Lund, Sweden, [3]University of Bristol, Bristol, United Kingdom, [4]The University of New South Wales, Sydney, Australia.
Biological motors are involved in various cellular processes such as intracellular transport, DNA replication and cell motility. These examples involve multisubunit proteins which transduce chemical energy into mechanical work. To understand better the underlying principles by which biological motors operate, it is instructive to study simpler motors which use Brownian diffusion coupled with asymmetry in the system to bias the direction of motion.
Here, we describe the design and construction of a novel protein-based synthetic motor, the ''lawnmower'', which uses a burnt-bridges mechanism to autonomously and diffusively move forward. The blades of the lawnmower are proteases bound to a quantum dot hub that interact with a one dimensional peptide substrate track via binding to and cleavages of the substrates. Simulations have suggested how the number of blades affects the motor properties: too many able to simultaneously bind the track means very slow motion; too few and the motor has low processivity [*Samii et al., Physical Reveiw E, 84, 031111 (2011)*]. In our design, cleavage of substrate by a protease releases a quencher molecule at one end of the peptide resulting in increased fluorescence of the DNA-bound product. Increased fluorescence thus acts as an indicator of the processivity of the lawnmower along the peptide track, which can be correlated to the motion of the lawnmower. This correlation provides an assessment of the directionality and processivity of our molecular motor and insight into its mechanochemical coupling. Experimentally, we confirm with kinetic assays that our lawnmower is active and that there are an average number of 8 blades on the each motor. We also demonstrate the synthesis and characterization of a highly modified DNA-peptide construct, which acts as the track for the motor.

2800-Plat
Synthetic Lipid Membrane Channels formed by Designed DNA Nanostructures
Martin Langecker[1], Vera Arnaut[1], Thomas G. Martin[2], Jonathan List[1], Stephan Renner[1], Michael Mayer[3], Hendrik Dietz[2], Friedrich C. Simmel[1].
[1]Lehrstuhl für Bioelektronik, Physics Department and ZNN/WSI, Technische Universität München, Garching, Germany, [2]Center for Integrated Protein Science at the Walter Schottky Institute, Technische Universität München, Garching, Germany, [3]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.
Scaffolded DNA origami was used to create a synthetic membrane channel that consists of a hollow stem that penetrates and spans a lipid bilayer membrane, and a barrel-shaped cap that adheres to the membrane via cholesterol moieties. Transmission electron microscopy was used to confirm that the intended shape is realized and that the synthetic DNA channels bind to lipid membranes in the desired orientation. The conductance of the resulting membrane pores was studied in electrophysiological experiments. Successful membrane incorporation of individual synthetic DNA channels manifested itself in a stepwise increase in transmembrane current and an increase in electrical noise. The DNA channels displayed an Ohmic conductance of ~ 1nS per channel in 1M KCl, which agrees with expectations based on the channel geometry. Similar to naturally occurring ion channels, the synthetic DNA channels display gating behavior, which may be caused by thermal fluctuations of the structure. Gating behavior is found to differ significantly for different variants of the channel. Geometry and chemical properties of DNA channels can be tailored for custom nanopore sensing applications. We also demonstrate that the channels can be used for single molecule studies of DNA secondary structures.



2801-Plat
Biomimetic Membrane Channels based on Carbon Nanotubes
Jia Geng[1,2], Kyunghoon Kim[2,3], Costas Grigoropoulos[3], Caroline Ajo-Franklin[2], Aleksandr Noy[1,4].
[1]UC Merced, Merced, CA, USA, [2]The Molecualr Foundry at LBL, Berkeley, CA, USA, [3]UC Berkeley, Berkeley, CA, USA, [4]Lawrence Livermore National Laboratory, Livermore, CA, USA.